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AUTHOR Holtzman, Neil A.
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ABSTRACT

This monograph, designed for persons involved in the organization and regulation of screening of newborns infants for Phenylketonuria (PKU) and other genetic-metabolic diseases reviews new developments in the field, makes recommendations, and provides information about specific conditions other than PKU that are detectable by screening. Discussed briefly in Part I are various aspects of screening for PKU, ethical and legal issues and screening for other conditions. Part II suggests a model for supervising screening operations for any disorder (from determining what conditions should be screened for, through implementation to evaluation); discusses laboratory responsibilities and offers specific recommendations regarding PKU screening. Part III lists conditions that can be screened for with four different procedures. (SB)

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NEWBORN SCREENING FOR GENETIC-METABOLIC DISEASES

U.S. DEPARTMENT OF HEALTH, EDUCATION & WELFARE
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Foreword

In 1966 the Department of Health, Education, and Welfare issued "Recommended Guidelines for PKU Programs for the Newborn" [1] to assist health departments and others in establishing screening services for the detection and care of newborn infants with phenylketonuria. Programs that now function in most States were initially based on these guidelines, and have been frequently modified to include new developments. But there are problems that must be addressed, particularly in light of new possibilities:

1. PKU screening is often of less than optimal (and attainable) effectiveness.
2. Parents are often poorly informed about screening and about the use of specimens and results.
3. Neonatal screening tests for other conditions are available but the question arises of when they should be used on a population-wide basis.

The first part of this monograph is a review of new developments related to these areas. Recommendations are presented in the second part. Part III provides additional information about specific conditions other than PKU that are detectable by screening.

This publication is intended primarily for those involved in the organization and regulation of screening as a service. As the objective "is to find affected subjects at a time when intervention may prevent the ill effects of the disease" [1], responsibility includes assuring that efficacious therapy will be effectively provided. Procedures involved in the actual diagnosis and management of phenylketonuria, which will be of interest to those providing care to infants with presumptive positive screening tests, are dealt with in other publications. [2, 3]

The Collaborative Study of Children Treated for Phenylketonuria (a joint effort of 15 clinical programs coordinated through Children's Hospital of Los Angeles and supported by the Bureau of Community Health Services through funds appropriated under title V of the Social Security Act) has collected a great deal of data on outcome of treatment of PKU as well as on screening. The author's evaluation of screening programs was initiated on behalf of the Collaborative Project.

Participants in the PKU Collaborative Project include:

Children's Hospital
Los Angeles, California

Regional Center for the Developmentally Disabled
Childrens Hospital and Health Center
San Diego, California

University of Colorado School of Medicine
Denver, Colorado

Mailman Center
University of Miami
Miami, Florida

Cook County Hospital
Chicago, Illinois

Children's Memorial Hospital
Chicago, Illinois

Child Development Clinic
University of Iowa
Iowa City, Iowa

University of Maryland School of Medicine
Baltimore, Maryland

Johns Hopkins Hospital
Baltimore, Maryland

Children's Rehabilitation Center
Children's Hospital
Buffalo, New York

State University of New York
Syracuse, New York

Children's Medical Center
Tulsa, Oklahoma

University of Texas Medical School
Galveston, Texas

University of Washington
Seattle, Washington

Waisman Center on Mental Retardation and Human Development
University of Wisconsin
Madison, Wisconsin

Part I: New Developments

PHENYLKETONURIA

Prognosis

There can no longer be any doubt that the early institution of a diet low in phenylalanine is efficacious in preventing retardation from phenylketonuria. The PKU Collaborative Project reports that the IQ (mean ± s. d.) at 4 years of age in 97 PKU children identified and treated as a result of neonatal screening was 92 + 15.1.[4] This is much higher than in untreated or late-treated phenylketonurics, including siblings of the study group. The best outcome was observed in those in whom the diet was started by 3 or 4 weeks of age.[4, 5] Since screening has become widespread, the admission of children with PKU to mental institutions has virtually ceased.[6]

Transient elevations and variants

Not all infants who have elevations of serum phenylalanine on screening have classical PKU. Eighty-five percent of infants with presumptive positive screening test results have normal concentrations on the next followup. Only 5 percent will eventually be proved to have PKU.[7] Most of the remainder with initial elevations will eventually have normal phenylalanine concentrations (less than 6 mg/100 ml), but between 1 and 2 percent will have persistent moderate elevations of up to 20 mg/100 ml of blood phenylalanine on regular diets.[8] These variants probably represent several different defects but most of them are not at risk of retardation.[9, 10] Restriction of dietary phenylalanine in a variant may result in a severe deficiency state and has no proven benefit. Thus, in any infant with a positive screening test, criteria compatible with a diagnosis of classical PKU must be present before therapy is started.[2]

A form of phenylketonuria causing severe retardation which is unresponsive to dietary restriction of phenylalanine has recently been reported.[11] In some of these infants the phenylalanine concentration may not rise excessively when they are challenged with a regular diet.[12] This form can be detected in fibroblasts and, as these infants may benefit from early treatment with other modes of therapy, skin biopsy for culture should be considered in any infant with persistent elevations of phenylalanine.

False negatives

Between 5 and 10 percent of infants ultimately proved to have phenylketonuria are not detected by newborn screening (false negatives).[7] Most of them are missed because of the early age at which they are screened; the blood phenylalanine in infants with PKU may not rise above the usual cutoff concentration until 4 days of life or later.[13] Although poor feeding contributes to the possibility of normal values in the first few days of life, there are other influencing factors as well.[14] In the United States most infants are screened on or before the fourth day of life.[7] When infants are screened later, as in the United Kingdom, there are virtually no false negatives.[15]

Laboratory error

Laboratories involved in screening do not always obtain the true phenylalanine concentration in specimens presented to them.[16] Errors which are marked around the usual cutoff range of 4 mg/100 ml explain the significant differences in the incidence of presumptive positive tests; States reported incidences between 5/100,000 and 275/100,000 in infants tested on the third day of life.[7] As the blood phenylalanine in infants with PKU may be only minimally elevated in the first 4 days of life, laboratory error contributes to the failure to detect some affected infants. The urine ferric chloride test for PKU, or the Phenistix® dip stick, will fail to detect a significant number of infants with PKU[17] regardless of their age. It should not be used as a screening test.

Delays

Excessive time is frequently required to retrieve an infant with an elevated first test. As a result of the delay some damage may be sustained by affected infants. In a nationwide survey, the average time between the first test and the followup was 25 days and in over 22 percent of infants, the time lapse was greater than 1 month to obtain a followup.[7] These delays could be shortened by reducing the number of screening laboratories and establishing well-defined procedures for communication between those collecting specimens, the laboratory analyzing the specimens, and those providing followup care.[15]

Unresolved problems

The successful management of PKU in early childhood has raised other problems, such as the question of when the diet should be terminated. The Los Angeles PKU Collaborative Project is studying the problem by terminating the low phenylalanine diet in one-half of its subjects, selected at random, at 6 years of age.[18]

A second problem relates to the observation that infants of phenylketonuric mothers, most of whom would not be expected to have PKU, are almost always retarded and may, in addition, have physical anomalies.[19] The question remains unanswered as to whether females with PKU, who have attained normal intelligence as a result of early treatment, can be protected from having retarded offspring by maintenance of a low phenylalanine diet during pregnancy.

ETHICAL AND LEGAL ISSUES

Failure to define objectives, benefits and risks, obtain consent, and assure confidentiality, particularly in chromosome screening and sickle cell carrier detection, has prompted concern for safeguarding individual rights in all genetic screening programs, including those for PKU. Recently, a committee of the National Academy of Sciences reviewed biomedical, legal, and ethical aspects of genetic screening and offered procedural guidance.[20]

Informed consent

Most laboratory tests are performed because the patient seeks help. In screening, however, it is the patient who is sought and many healthy subjects are perturbed. Consent for screening, therefore, must be viewed differently than consent for tests that result from patient-initiated contact. Informed consent need not be a barrier between the screener and the patient and could, in fact, eliminate misunderstandings. Provision of information to parents before their infant is screened for disorders for which treatment is available could allay anxiety at the time of the test and result in greater willingness of the parents to bring the baby in for a second test, should that be necessary. If, however, the requirements for informed consent result in significantly fewer babies being screened, a mandatory program might be legally justified under the *parens patriae* doctrine which enables the State to act to protect those who cannot protect themselves.

Any use of a specimen for purposes other than the demonstrated benefit of the infant being screened constitutes research and must conform to established guidelines for research in children.[21] The doctrine of *parens patriae* would not apply when proof is lacking that the screening test affords protection to the infant. Thus, in situations when a specimen (or part of it) will be used to validate a new test or to identify infants in whom a new treatment will be tried, failure to inform the parents about all aspects of the procedure and to obtain consent cannot be justified.

Right to information

In newborn screening programs it may not be feasible to inform parents of normal test results. This is legally permissible provided that parents are

advised of it in advance and agree. Investigators may also wish to withhold results in situations when the specimen (or part of it) will be used to identify infants with conditions whose natural history is uncertain. Infants may be recruited into such studies if their parents are informed in advance and given an opportunity to refuse to allow them to participate.

Except for statistical data compiled without reference to the identity of the subjects, information regarding test results should not be released (for example, to school authorities) without the consent of the infant's parents. There is no threat to the public health—as in the case of communicable diseases—to justify release of the information. Furthermore, release of the information may lead to unwarranted stigmatization of the infant or his family.

Regulation of screening

As it becomes possible to screen for additional disorders, legislators may be influenced to pass laws dealing with each of them. State laws now exist dealing with sickle cell screening, histidinemia, galactosemia and other conditions. Recognizing that separate laws for each new disease may be cumbersome and may not reflect the best interests of its citizens, the Maryland State Legislature created a Commission on Hereditary Disorders which has the authority to promulgate regulations and standards for the detection and management of genetic conditions in the State. As a result of having staggered terms of office, the Commission always has experienced members on it. Consequently, it is in a better position than the legislature, to which it is responsible, to evaluate the pros and cons of screening for specific disorders. The inclusion of consumer members provides greater assurance that programs recommended by the Commission serve the best interest of the public and that rights of informed consent and confidentiality are safeguarded.

Regionalization

In some States the number of births per year may be too small to support an efficient screening program and several States may contract with one center to provide laboratory services and clinical consultation. A regional network of screening covers Alaska, Montana, and Oregon and a similar network includes Maine, Massachusetts, and Rhode Island. Regional networks also exist in the Province of Quebec, Canada,[22] and in several other countries.

The Secretary of Health, Education, and Welfare has suggested the establishment of regional networks for laboratory work related to prenatal diagnosis.[23] If this were to occur, newborn screening tests might also eventually be performed in such regional laboratories. This would improve efficiency and minimize delays in followup provided that procedures were established concomitantly for the rapid transmission of specimens and results to and from the regional laboratory.

Screening For Other Conditions

Newborn screening tests have now been reported for over 20 disorders.[24] Most of them require specimens from infants who are a few days old, as they measure metabolites whose accumulation in affected infants is, at least in part, dependent on dietary intake. Cord blood can be used for enzyme or protein assays to detect hypothyroidism[25] and galactosemia.[24] It has also been used to identify women with phenylketonuria as the infants of such mothers have high phenylalanine concentrations in cord blood but not in specimens obtained later.[24] Amino acid chromatography of blood, particularly when obtained in the second week of life, enables detection of PKU and other abnormalities with one test.[26] Urine chromatography on specimens collected at 2 to 4 weeks of age has also proved helpful in detecting conditions that may be missed by blood screening at an earlier age.[24] Part III of this publication provides additional information on a number of conditions.

Availability of a test is insufficient reason for instituting a public screening program. Some of the chemical disorders which are detected are not associated with any disease state. For others, the relation is uncertain and further study is needed. Also, many tests require further validation. As a minimum condition newborn screening should meet at least one of the following criteria before it is offered as a routine service: 1) Affected subjects who are discovered will benefit, as in the case of PKU, from the early institution of therapy; 2) affected subjects and their families will be apprised of hazardous situations which should be avoided (e.g., genetically determined drug and food intolerances); 3) the families of affected subjects discovered to have generic disorders can be counseled about risks of recurrence.

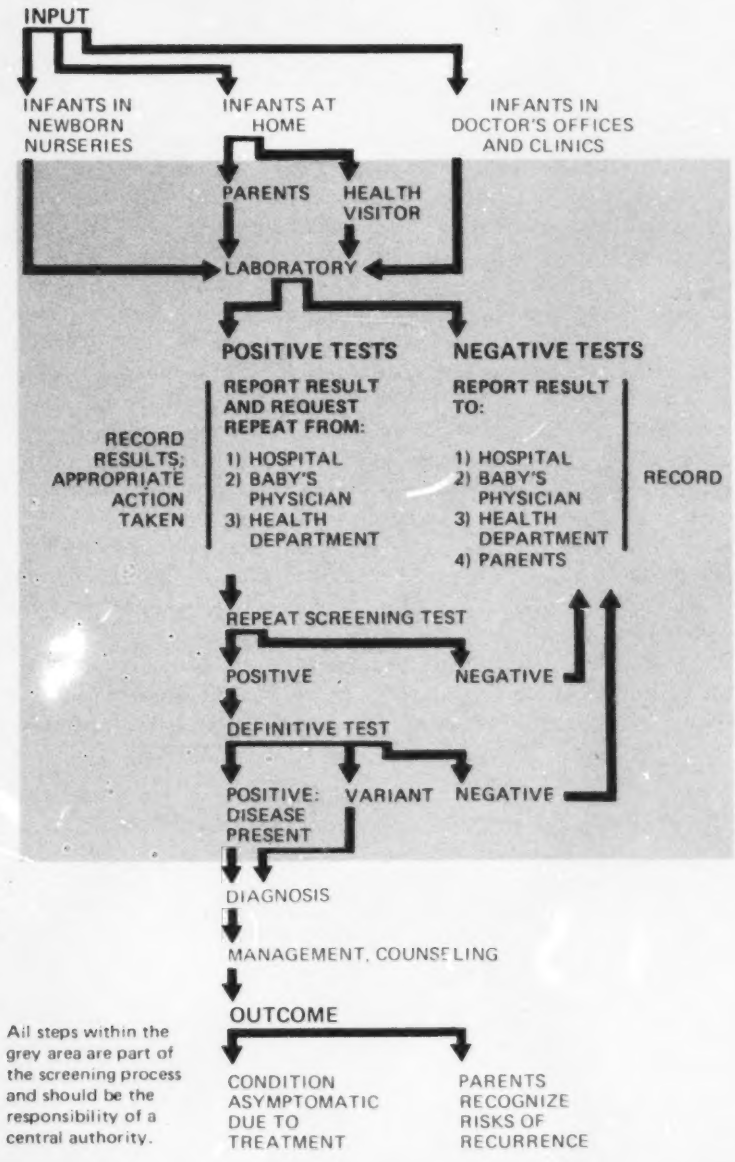


Figure 1 – SCREENING: PROCESS AND OUTCOME

Part II: Recommendations

The first section of this part suggests a model for supervising screening operations for any disorder, from innovation, through implementation, to evaluation. The second section deals with laboratory responsibilities and is intended not only for laboratory personnel but for those in the screening authority to whom the laboratory is responsible. The third section offers specific recommendations regarding PKU screening.

A CENTRAL ORGANIZATION FOR SCREENING

Screening is more than a laboratory service. (See fig. 1) It involves many persons and operations from the time the infant enters the screening process, continuing through the laboratory procedures, until diagnosis is made and management of the condition is decided upon.

An administrative organization such as a State central authority under a State Health Department, or a regional grouping of State Health Departments, could most easily assure efficient operation and acceptance of a coordinated comprehensive program encompassing the activities identified in figure 1. The proposed authority would work with an advisory board consisting of: 1) A representative of hospitals who send specimens; 2) a specialist in metabolic disease; 3) a nurse and/or nutritionist involved in management of patients; 4) a representative of practicing physicians; 5) the director of the laboratory performing the tests; and 6) consumers.

This proposed central organization could have the following responsibilities:

Determine which conditions should be screened for

This decision should be based primarily on the following considerations: 1) Frequency and severity of the condition; 2) availability of a therapy of proven efficacy; 3) extent to which detection by screening improves the outcome; 4) validity, reliability, and safety of the screening tests; 5) adequacy of resources to assure effective screening and followup; 6) costs; and 7) acceptance of the screening test by the community, including both consumers and practicing physicians. It must be emphasized that the availability of a test is an insufficient condition for undertaking routine screening.

Determine procedures for collection of specimens

The age of the infant at the time of the test is most crucial and will depend on the specific disorder under consideration. Once the time is decided, responsibility for collection of specimens must be assigned and accepted. Collection of specimens during the first few days of life is usually performed by hospital personnel but in the second week or later it can be done in the home by health visitors or parents (when urine is needed), in a clinic or office, or in the hospital on a return visit. Standardized instructions, test forms, mailers, and other needed materials should be provided to all who collect specimens. Situations have been uncovered in which hospitals decided which filter paper to use for the collection of blood specimens and errors occurred because the wrong material was selected.

Determine procedures for transmission of specimens and results, recording of data

All steps up to and including referral of infants with presumptive positive tests should be performed with sufficient rapidity so that those in whom the diagnosis is established receive maximum benefit. To help assure that the test is performed on every infant for whom the parents consent, a notation on the hospital medical record or birth certificate should be required.

Determine adequacy of laboratory (or laboratories) in the State (See also Laboratory Responsibilities)

The central organization should be able to request the laboratory to evaluate tests for additional conditions, document periodically the adequacy of tests already in routine use, and use specified procedures to report results.

Referral center

Any center to which infants with presumptive positive tests are referred should be capable of confirming the diagnosis, initiating and monitoring therapy, and counseling the families. In order to accomplish this the center should have experience with the disorder, or related ones, and should have defined lines of communication to the central authority and screening laboratory on the one hand and to practicing physicians who provide primary care to the patients with these conditions on the other. The central authority should assure that no baby discovered by screening to have a genetic disorder be denied treatment for financial reasons.

Education and information

Physicians, colleges and schools, and the mass media should be utilized to educate the community regarding the availability of and reasons for screen-

ing. Information should be provided to pregnant women and their partners about the objectives of the test, when and how it will be done, which results will be transmitted, and how. In cases of presumptive positive and unsatisfactory screening results, the central organization might make available form letters that could be used to request followup tests. Practicing physicians must be kept informed about which conditions are being screened and limitations of the tests.

False negatives are possible with any test. In older infants with suspicious findings the diagnosis should not be ruled out on the basis of a negative screening test.

Determine informed consent procedures

To obtain consent, information may be presented to the parents either prenatally or after birth, but not when the mother is in labor or immediately postpartum. A statement may be included that unless a parent specifically objects, the test will be performed. A more positive consent may be obtained orally, noted on the infant's record, or still more formally, by written statement. If part of the specimen will be used for investigational purposes, the consent should note this intent and describe the type of investigation. A brochure providing information about the test(s), as well as a standard consent form, should be distributed by the central organization.

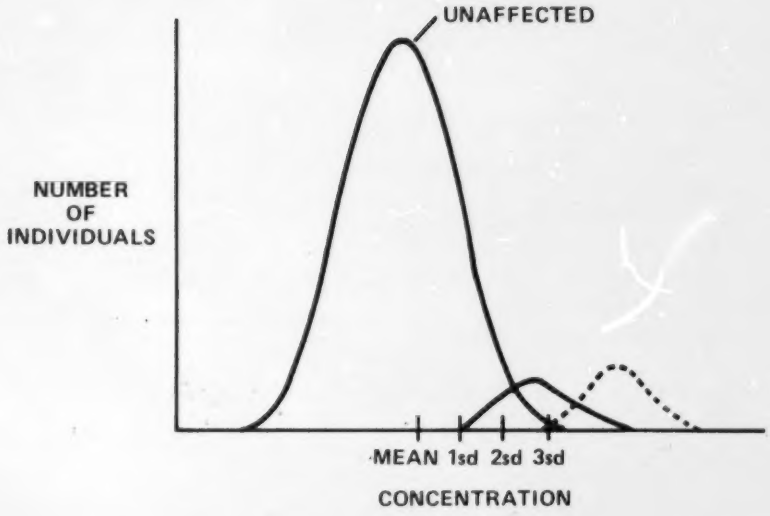
Evaluation

The central organization should periodically evaluate the individual components of the program as well as the entire program. (See Evaluation under section, Recommendations for Screening for Phenylketonuria).

LABORATORY RESPONSIBILITIES

Quality control

Because of problems in the performance and interpretation of screening tests, specimens should be analyzed in a large central facility. This may be one laboratory within a State or, when the number of births in a State is too small to permit efficient utilization, one laboratory serving several States. If more than one laboratory must be utilized within a State, the health department must rigorously check the quality of each laboratory. In addition, to provide equal coverage of all infants born in the State, all of the tests deemed routine by the central authority should be performed by all laboratories that are authorized to perform tests.



The small curves represent affected infants; the solid line, their results in the first week of life, the dashed line, their results a few weeks later. sd = standard deviation for the mean of all test results during the first week of life. To detect all affected infants during the first week would require repeat testing of all infants who exceeded the mean by one sd. At the later age repeat testing would be be needed only on those who exceeded the mean by 3 sd. Test results in unaffected subjects are much less affected by age. The diagram also applies when affected infants have reduced, rather than elevated, concentrations of the compound being screened.

Figure 2 – DISTRIBUTION OF TEST RESULTS IN A POPULATION SCREENED DURING THE FIRST WEEK OF LIFE

The Center for Disease Control, Atlanta, Georgia, will provide standard specimens that can be used to check the reliability of methods in each participating laboratory, assuring consistency of results between laboratories. Bacterial assays and chromatogra[illegible]equire visual comparisons of an unknown and a standard. To minim[illegible]e error, standards should be included in the daily runs and, from time to time, specimens with elevated concentrations inserted as unknowns. Intra- and inter-observer variability should also be checked.

The possibilities of error are too great to ever base a diagnosis on a single test result; this is true of any laboratory test but particularly of screening tests in which the procedure itself, as well as the number of specimens being handled simultaneously, increases chance of error. Thus, a confirmatory test on a second specimen is always part of the routine management of positive screening tests.

Changes in supplies, personnel, instruments, or climatic conditions can alter the results of tests routinely performed. Consequently, there is need to review periodically results obtained and to alter procedures or cutoff points accordingly. Methods reported in the literature should be followed closely but cutoff points should not be accepted without validation on a sample of the local population which may differ in age or in environmental (e.g., nutritional) or genetical factors from the population on which the literature report was based. Handling of the specimens may also differ.

Determination of the cutoff point and optimal age for testing

A cutoff point which distinguishes all of those with *overt manifestations* of a condition from those who do not have the condition may not distinguish all, or even most, *presymptomatic* infants from those without the condition. In affected subjects in the immediate postnatal period in particular, the concentration of substances destined to reach much higher levels may be only minimally elevated.

Ideally, in order to determine how frequently the cutoff level that is selected will miss affected infants, another test should be performed in every infant: Either a more definitive method (for example, quantitative determination of enzyme activity when the screening test measures metabolite concentration) should be employed simultaneously, or the test should be repeated when the infants are older. (Alternatively, all infants should be examined for the condition at an age when clinical manifestations are usually present; this may be several years after they are tested.) If the second test reveals infants with the condition who had levels below the cutoff point on the screening test, either the cutoff point should be changed or the test should be performed when infants are older.

As the cutoff point approaches the mean of all test results, more and more subjects who do not have the condition will exceed it (fig. 2). A large number of such "false positives" inflates the cost of screening as they must all be followed up. Thus, rather than alter the cutoff point it may be more efficient to screen infants when they are somewhat older, provided therapy will still be effective.

A less satisfactory method of determining an optimal cutoff point than performing two tests on *all* infants involves performing second tests only on those whose screening test results fall outside of some multiple of the standard deviation of the mean on the abnormal side (fig. 2). For conditions of low incidence a higher multiple of the standard deviation can be selected in order to increase the ratio of true to false positives among those receiving a second test. This approach assumes that all subjects with the condition fall within the population on which another test is performed.

For tests which are already in routine use, marked changes in the mean or standard deviation in the most recent period compared to the past may suggest the need for revalidation. If the incidence of the condition is lower than that reported on comparable populations then revalidation is also indicated.

Storage of specimens

Specimens should be saved, under conditions to maximize stability, for as long as possible. Phenylalanine and some other amino acids in dried blood spots on filter paper are stable for several years. When an infant whose screening test was reported as negative is discovered to have the disease for which screening was performed, a retest can indicate whether the laboratory was in error. Saved specimens may be used for validating tests for other conditions, provided parental consent was given. Should a serious disease be shown to have a biochemical aberration, analysis of the specimen saved from affected infants may indicate that the condition is detectable in the newborn period.

Recording of results

The laboratory should keep permanent records of all results and periodically tabulate them in order to determine changes in the frequency of both true and false positives. The interval it takes for each hospital to send specimens should be checked periodically and delays corrected. A system should be developed to flag incomplete records on which a followup is due.

Records of all results are confidential and should be released only to the hospital responsible for the collection of the test. Test results may be placed on the infant's medical record, or reported to the baby's physician or other person specifically agreed to by the parents.

Evaluation of tests for new conditions and new tests

The consent of the parents is required, unless the specimen is used anonymously, to establish the precision and reproducibility of new methods. When a new test is being considered for a condition for which screening is already routine, it should be run simultaneously with the old on the same specimens.

RECOMMENDATIONS FOR SCREENING FOR PHENYLKETONURIA

Benefits of PKU screening are sufficiently well established to recommend testing every baby born in this country. Since there is not enough experience with other conditions to make blanket statements about the advisability of screening for them, each authority should be the judge of the capability of its jurisdiction to screen for additional conditions. Screening for a number of other conditions is reviewed in Part III of this publication.

Information and consent

Every pregnant woman should receive information concerning PKU screening. The central authority should distribute to obstetricians, prenatal clinics, and others responsible for the care of pregnant women a brochure containing the following information: 1) Definition of PKU and reasons for the test; 2) indication that the test will not give information about other causes of mental retardation; 3) description of the test procedure including when and where it will be performed and how the specimen will be obtained; 4) description of the risks including the approximate number of tests already performed and the number of adverse reactions, or the lack of them; 5) reasons that followup will be requested explaining that a positive screening test does not, by itself, indicate the presence of phenylketonuria; and 6) description of how the results will be communicated. If negative results will not be communicated the parents should be so informed. (They should be told how they can obtain the results if they want them.) Parents should also be informed of the time it will take for notification of positive results, or if the test must be repeated because the specimen was unsatisfactory.

It may be appropriate to include information on PKU screening with information concerning other recommended procedures (e.g., typing for blood incompatibilities, Tay-Sachs testing in the Jewish population, and sickle cell screening in the black population). Information on PKU can also be communicated, perhaps for a second time, after the woman delivers her baby. If a parent refuses to consent to the test, the refusal should be recorded in the infant's medical record.

Timing of the test

Specimens should be collected no sooner than 24 hours after the onset of milk feeding and as close as possible to nursery discharge, but no later than 14 days after birth.

If most infants are discharged on or before the fourth day of life it is preferable to screen in the second week of life. However, unless there is assurance that this can be accomplished for *every* infant, routine screening prior to nursery discharge should be performed as recommended earlier.

A second blood test between 1 and 4 weeks of age is recommended for all infants screened on or before the fourth day of life, or in infants with vomiting or feeding difficulties prior to the time that the first test was collected. If a second test is routinely performed in order to detect other disorders, then a repeat test for phenylalanine should be performed at that time on all specimens.

Infants born into families with a history of PKU should be tested daily during the hospital stay after birth and, if the results remain negative, the test should be repeated again at 2 weeks of age.

In premature infants the specimen should be collected not later than 14 days after birth or as soon as protein intake is adequate.

Responsibility for testing

The responsibility for collection of the specimen rests with the person in charge of the institution in which the child is born (or his designated representative) or, if a child is born outside the institution, with the person delivering the infant or required by statute to file the certificate of birth. If an infant is discharged from the institution sooner than 24 hours after the onset of milk feeding or before a blood test is collected, the institution should be responsible for ensuring that a satisfactory test is obtained by 2 weeks of age. If tests are to be performed routinely after babies are discharged from the hospital, the responsibility can be assigned to the baby's physician or well baby clinic, to a health visitor in the home, or to the hospital in which the baby is born by requesting the parents to return at a specified time.

Collection of specimens

The following information should be affixed to each specimen: Name, sex, race, date of birth, identifying number of the infant, time and date of the first feeding, and time and date at which the specimen was obtained. Birth weight, type of feeding (breast or bottle), volume of milk ingested in last 24 hours, condition of the baby at discharge, and name of the baby's physician are other useful items. Supplies for the performance of the test should be provided by the central authority.

All specimens should be forwarded by first class mail to the laboratory within 24 hours of collection. The envelopes in which they are mailed should be marked or colored to indicate that they require prompt handling.

Report of test results

The laboratory should report test results to the institution or individual responsible for the collection of the specimen within 5 days after its receipt. In cases of positive tests telephone communications may be used as well.

The report of each test should be entered on the infant's medical record and, in the case of negative tests, be available to parents on request. No test result should be released by the laboratory, the institution, or any other individual, except as already described, without the written authorization of the parent.

Repeat tests for presumptive positives

In case of a presumed positive test, the institution or individual, or his designate, responsible for collection of the screening specimen should be responsible for obtaining a repeat test and should do so promptly. If the infant cannot be retrieved for a second test, the central authority should be notified and further followup attempted by the local health department. Care should be taken not to alarm the parents unduly, particularly when a second test is requested because the screening test was unsatisfactory. The family's physician should be notified whenever possible. Parents should be notified of the results of followup tests, even if negative, so that they do not remain concerned.

The central authority should be notified by the laboratory of confirmed positive tests so that the authority may inform the institution or the individual who collected the specimen as well as the family's physician. The central authority must also assure referral to a center experienced in the field of genetic-metabolic disorders for confirmation of the diagnosis and management of the treatment. The infant and family will benefit from the establishment of a cooperative relationship between their physician and the consultant.

Criteria for diagnosis of PKU

The HEW publication "Management of Newborn Infants with Phenylketonuria" [2] contains a detailed description of diagnostic procedures and management. In order to establish a diagnosis of PKU once a positive test is obtained, at least two separate determinations by a quantitative method should yield a blood phenylalanine concentration of 20 mg/100 ml or higher and a tyrosine concentration of 5 mg/100 ml or less. Both of these specimens should be collected when the baby is receiving adequate amounts of regular formula. If these criteria are satisfied, dietary phenylalanine should be restricted even if the ferric chloride test is negative. (More sensitive procedures may indicate the presence of phenylalanine metabolites in the urine.) After the infant is started on a low phenylalanine diet, frequent monitoring of serum or blood

phenylalanine concentration is needed. Lower than expected levels suggest the possibility of a variant form. In any event, the diagnosis should be confirmed by 6 months of age by challenging with a formula containing normal amounts of phenylalanine.

Acceptable screening procedures

Laboratory methods for screening have been reviewed elsewhere.[8, 24, 26, 27, 28] The Guthrie bacterial inhibition assay, the McCaman and Robins fluorometric assay, and chromatographic procedures on either paper or thin layer plates are screening methods currently in use. The fluorometric method is most sensitive and precise and paper chromatography is the least sensitive. Paper chromatography should not be used when the specimen is collected in the first week of life. Determination of the cutoff value by any of these methods should be made in each laboratory according to the procedures described in the preceding section on Laboratory Responsibilities. Neither the Guthrie bacterial inhibition assay on urine nor the ferric chloride test nor Phenistix® test is a suitable screening test at any age.

Evaluation

In order to evaluate the screening program, once each year each hospital should report its number of births, initial and repeat tests, and refusals to the central authority. The laboratory, or laboratories, performing the tests should report the number of first and repeat specimens received, and the average and median time from collection of the specimen to receipt by the laboratory for each hospital. The laboratory should also report the time required to process specimens (the time between receipt in the laboratory and the mailing of results) and the interval between the first positive test and followup. Disposition of all infants with positive tests should be determined from appropriate sources by the central authority and the incidence of PKU, variants, and presumptive positive tests should be calculated and compared to similar data from earlier years and other regions.

On the basis of this information the central authority responsible for the entire screening program can suggest appropriate modifications of procedures by the hospitals, laboratories, or others involved in the program.

Part III: Tests for other disorders

Over 20 different genetic-metabolic conditions may be detected by analysis of meconium, umbilical cord blood, blood in the first few weeks of life, or urine at 2 to 4 weeks of age. Although each condition represents a chemical aberration, some are not responsible for disease—iminoglycinuria, for instance. In others, such as histidinemia, Hartnup's syndrome, hypercholesterolemia, or cystinuria, it is doubtful that clinical problems will develop in every subject left untreated.

Infants with some of the other chemical abnormalities will, however, frequently go on to develop severe, overt disease (sickle cell anemia, thalassemia, some forms of homocystinuria, homozygous alpha$_1$ anti-trypsin deficiency, cystic fibrosis) for which, at the present time, there is no effective prophylaxis. Although neonatal detection of cystic fibrosis or sickle cell anemia may provide a family with information to avoid the birth of other affected children, counseling services are needed and the effectiveness of neonatal programs with this objective remains to be proven. Furthermore, neonatal screening for cystic fibrosis, using meconium, sweat, or other methods, requires further validation; a significant proportion of affected infants is being missed.[29] Screening of young adults for sickle cell carriers is a more effective way of offering reproductive information to high risk couples than neonatal screening.

Since some other detectable conditions are both severe and treatable but extremely rare, the cost of establishing routine screening programs just for them might be excessive. When, however, a screening program (including appropriate followup) already exists, when no additional specimen is required, when the methods required are similar to those in use, and when treatment is effective only when started early in life, the added cost may be small. Thus, while maple syrup urine disease and galactosemia are both rarer than PKU, they meet all of these conditions if PKU screening is in operation. However, both disorders can be fulminant and unless screening is done in the first week of life and results reported promptly, tests for them may be of no avail. The test for orotic aciduria in the newborn has not been validated because of its extreme rarity. The condition may also be treated effectively later in life if the diagnosis is made. It should be suspected in cases of refractory hypochromic anemia.

Finally, there are conditions detected in newborns which will result in difficulty only in specific environmental situations. Whether morbidity and

mortality from glucose-6-phosphate dehydrogenase deficiency or from hereditary angioneurotic edema will be reduced as a result of neonatal screening remains to be established.

The section that follows lists a number of conditions for which it *may* be of benefit to screen in specific testing circumstances. Either treatment is available or the family will benefit from counseling. In each case the decision to undertake screening should be based on the availability of resources at all stages in the process (fig. 1) as well as on validation of the procedure in the local laboratory.

A. Dried blood on filter paper collected in the first week of life:

1. Phenylketonuria
2. Maple syrup urine disease
3. Galactosemia

Bacterial assays for metabolites which accumulate are available for each of these conditions.[24] An enzymatic assay for the severe, classical form of galactosemia is also available but exposure of specimens to heat (e.g., warm weather) or dampness (e.g., not air-dried before mailing) leads to inactivation of normal enzyme.

4. Hypothyroidism

Recently, a screening test for thyroxine (T_4) was reported capable of detecting congenital hypothyroidism on the fifth day of life.[30] If performed earlier the test may fail to detect some infants with deficiency. There are many false positives; 1 percent of infants require followup. The T_4 test is less sensitive than the TSH assay (see C2) performed on cord blood, and very recently on dried blood on filter paper. Hypothyroid infants benefit from early treatment.

5. Tyrosinemia

Except in populations in which hereditary tyrosinemia has been observed, this condition is probably too rare to merit routine screening. One percent of all infants may have transient elevations of tyrosine,[24, 26] often accompanied by moderate phenylalanine elevations. In a specimen with an elevated phenylalanine the finding of an elevated tyrosine makes a diagnosis of PKU unlikely and reduces the number of infants who need rapid followup to ascertain the presence of PKU.

Fluorometric as well as microbiologic assays for phenylalanine, tyrosine, and galactosemia are available.[22]

B. Dried blood on filter paper collected from the beginning of the second to the fourth week of life:

1. Phenylketonuria
2. Maple syrup urine disease
3. Galactosemia

It may however, be too late to screen effectively for galactosemia and maple syrup urine disease.

4. Hypothyroidism
5. Tyrosinemia
6. Homocystinuria

The bacterial assay will only detect those forms of the disorder in which serum methionine is elevated. In the first week of life the test may not be sufficiently sensitive to detect the form responsive to vitamin B_6[24] and even at a later age further validation is needed. Certain other inborn errors as well as high protein intake may also cause elevations of methionine. Phenylketonuria, maple syrup urine disease, tyrosinemia and homocystinuria can be detected by either paper or thin layer chromatography.[26, 27] With current stains the method may be less sensitive than those previously mentioned and, consequently, it should not be used in infants younger than 1 week without careful validation.

7. Hyperglycinemias

At the present time chromatography is the only routinely used screening method theoretically capable of detecting conditions in which hyperglycinemia is present. However, blood levels may be only minimally elevated and affected infants could escape detection. Infants with all but the nonketotic form appear to benefit from treatment. Genetic-metabolic disease should be considered in any young infant with ketoaciduria. Amino acid chromatography is also likely to discover unknown compounds or elevations of known amino acids which have not hitherto been associated with disease. If the infants from whom such specimens are obtained are asymptomatic, no therapy is indicated but pathological changes may appear and these infants should be followed.

C. *Cord blood:*

Blood from the placental end of the umbilical cord can be collected into a test tube or onto filter paper. Care must be taken not to contaminate the specimen with maternal blood.

1. Maternal PKU

Most women of childbearing age are too old to have been screened in childhood. Thus, they may have undetected PKU. In such cases the cord blood will show an elevated phenylalanine, but the concentration will fall in the next few days of life unless, because the father is a carrier, the child has PKU. Even those infants of mothers with PKU, who do not themselves have PKU, will be retarded because of exposure to elevated phenylalanine in utero. Little can be done to benefit these infants, but the mothers can be counseled concerning risks to future offspring.

2. Hypothyroidism

A screening assay for TSH in cord blood has been reported which appears capable of detecting infants with hypothyroidism.[25] In order to assure that

affected infants will not be missed by either the TSH assay nor the thyroxine (T_4) method, an independent measurement (for instance, the other test) should be obtained. Or, all infants with negative tests should be followed up until the procedure is validated. (See A4)

3. Galactosemia

The enzyme assay allows early detection, facilitating therapy of the classical form.

4. Arginino-succinic acidemia

A screening enzyme assay has detected one infant [24] in the newborn period. The disorder is of variable severity. Prompt diagnosis of the neonatal form may be beneficial, although the outlook at the present time is gloomy. Treatment of the less severe form is probably efficacious in reducing mental retardation.

D. Urine collected between 2 and 4 weeks of age:

Parents can be given a filter paper kit, mailer, and instructions for urine collection [24] or the specimen can be collected at the time of the first office or clinic visit. This may be more costly in time than collecting blood. Fewer than 70 percent of parents may send in specimens. Chromatography of the urine can result in the detection of:

1. Phenylketonuria
2. Maple syrup urine disease
3. Tyrosinemia
4. Homocystinuria

This method detects both high and low methionine forms as homocystine can be directly observed.

5. Hyperglycinemia

Quantitative amino acid analysis of blood is needed to assure that hyperglycinemia as well as hyperglycinuria is present. Iminoglycinuria is a benign condition in which blood glycine is normal.

6. Arginino-succinic acidemia
7. Galactosemia

Although a generalized aminoaciduria may be present in this and other conditions at 1 month of age, chromatography of sugars is the specific urine screening technique.

8. Cystinuria

Most infants will never require therapy, but when there is a family history of renal stones further study to determine whether there is a need for prophylaxis is indicated.

Many other conditions can be detected by chromatography for amino acids but either their significance is unknown, no treatment is available, or they are not associated with disease. The screener should inform parents prior to the test about the possibility of detecting conditions in these categories.

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